Tracking Bacterial Pollution Sources in The Great Bay Estuary Watershed 2004

A Final Report to the
New Hampshire Coastal Program

Submitted by

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Introduction

The New Hampshire Department of Environmental Services and the University of New Hampshire have been working together since 1999 investigating pollution sources in estuarine and coastal waters using a microbial source tracking technique called ribotyping. The method allows researchers to identify the bacterial pollution by source category such as agricultural, human, wildlife or pet by comparing the genetic information found in the bacteria of polluted water with the genetic information in a ribotyping library. This source specific information is used to remediate pollution sources through site-specific elimination or reductions. For example, managers responded to a ribotyping study that showed bacterial pollution from human sources (Jones & Landry, 2003) by increasing boat pumpout access for recreational boaters and increasing septic system maintenance education in the study watershed.

The New Hampshire Coastal Program (NHEP) has a vested interest in remediating coastal pollution including sources that impact shellfish harvesting. The NHCP staff address pollution sources in a variety of ways including investigations of bacterial pollution sources. In 2004, the NHCP staff began discussions with the New Hampshire Department of Environmental Services (DES) Shellfish Program staff to work cooperatively on a research project that would identify bacterial pollution that was impacting shellfish growing waters. DES is responsible for the sanitary quality of the State's shellfish growing waters under the authority of RSA 143:21 and 143:21-a. The DES Shellfish Program classifies growing waters in accordance with the National Shellfish Sanitation Program (NSSP) guidelines and standards.

Among the varied activities of the Shellfish Program, the main tasks include routine water monitoring, paralytic shellfish poisoning monitoring, shellfish tissue testing and pollution source identification and evaluation. This study was conducted in response to the pollution sources identified by the Shellfish Program and deemed significant in terms of impacts to safe consumption of shellfish in the Great Bay Estuary watershed. Nine pollution sources were investigated using ribotyping. This method allowed for the identification of source species, which increases the potential for successful elimination of the sources that are impacting shellfish growing waters.

Project Partners

This project involved several state programs and the University of New Hampshire. The DES Shellfish Program and Watershed Assistance Program staff worked together to identify priority pollution sources for investigation. DES staff worked with the NHCP and researchers at the UNH Jackson Estuarine Laboratory to design the study elements and prepare cooperative agreements. NHCP and DES staff collected water and fecal samples throughout the study during both wet and dry weather conditions. The

NHCP provided the funding for the laboratory analyses and reporting. UNH conducted the bacterial and ribotyping analyses and interpreted the data for use in this report.

Project Goals and Objectives

The goal of this project was to determine the bacteria source species from nine of the priority pollution sources in the Great Bay Estuary as identified by the DES Shellfish Program. Specific objectives were as follows:

1. Collect water samples at the nine selected sites during both dry and wet weather depending on the condition(s) under which the source was deemed significant.
2. Analyze the water samples for bacteria concentrations and determine source categories using ribotype profiling.
3. Issue a report that describes the source species for each site and recommends remediation actions.

Methods

The following section describes the sampling site selection and provides details for field and laboratory methods used for this study.

Sampling Site Selection

All data from DES Shellfish Program shoreline surveys conducted in the Great Bay Estuary were compiled and reviewed for inclusion in this study. The data represented the bacteria levels at sampling locations under both dry and wet weather conditions. The initial data review screened for sites that had high fecal coliform concentrations in dry weather with an emphasis on direct sources of pollution to shellfish waters that are open for harvesting. Sites that were described as pipes (e.g., storm drain outfalls, straight pipes, etc) identified through this initial screening process were eliminated from the study because DES staff would investigate the pipes with dry weather flow under another program, namely the illicit discharge detection program in the Watershed Assistance Section. In addition, sites in the Little Harbor watershed were eliminated from this study based on the fact that another MST study for that sub-watershed was already underway. The remaining sites from the screening process included seven tidal creeks, streams and other sources that showed high fecal coliform concentrations in dry weather. Each of these seven sites was selected for this study. Additionally, two other sites were selected based on an ongoing problem site (GBPS001) in both wet and dry weather conditions and a site that showed elevated levels during wet weather (GBPS063). This brought the total number of sampling sites to nine. Table 1 identifies the sampling sites and provides descriptions.

The sites are located throughout the Great Bay Estuary watershed including Great Bay, Little Bay, the Winnicut River and the Bellamy River. The majority of sites (n=6)

are situated in Great Bay in the towns of Durham, Greenland and Newington. The Little Bay site is in Durham. The Winnicut River site is in Greenland. And the Bellamy River site is in Dover. Locations of the sites are shown in maps found in Appendix A. Four site location maps are provided. The first illustrates the general location of the sites and the following three show a closer view of the sites.

Table 1 Great Bay Estuary sampling sites, descriptions and weather conditions of concern.

Station ID	Waterbody Name	Town	Watershed	Source Type	Weather Condition of Concern	Latitude	Longitude
BLMPS017	Unnamed	Dover	Bellamy River	Perennial Stream	Dry	43° 9’ 24.66”	-70° 51’ 35.46”
GBPS001	Pickering Creek	Greenland	Great Bay	Perennial Stream	Dry and Wet	43° 3’ 7.02”	-70° 50’ 8.4”
GBPS014	Crommet Creek	Durham	Great Bay	Perennial Stream	Dry	43° 5’ 53.34”	-70° 53’ 0.6”
GBPS044	Unnamed	Greenland	Great Bay	Perennial Stream	Dry	43° 3’ 20.46”	-70° 53’ 15.54”
GBPS063	Foss Brook	Greenland	Great Bay	Drainage culvert under railroad tracks	Dry and Wet	43° 2’ 39.36”	-70° 51’ 3.78”
GBPS064	Shaw Brook	Greenland	Great Bay	Perennial Stream	Dry	43° 2’ 37.8”	-70° 50’ 52.14”
GBPS082	Unnamed	Newington	Great Bay	Tidal Creek	Dry	43° 4’ 4.62”	-70° 50’ 10.14”
ULBPS028	Unnamed	Durham	Little Bay	Intermittent Stream	Dry	43° 7’ 2.46”	-70° 52’ 24.49”
WINPS001	Unnamed	Greenland	Packer Brook and Winnicut River	Tributary to the Winnicut River	Dry	43° 2' 25.44"	-70° 50’ 12.12”

Fecal Material Selection

Based on the land uses and suspected pollution sources, the following animals were targeted for fecal material sample collection in this study:

Buffalo	Human (septic system)
Ox	Human (municipal sewer)
Horse	Deer
Otter	Cow

Field and Precipitation Methods

The following section describes the methodology used for collecting water and fecal samples during this study. All samples were taken in accordance with the DES *Generic Quality Assurance Project Plan for Microbial Source Tracking* (Landry, In Review) standard operating procedures.

Water Samples

Each sample was collected using a sterile Whirlpak™ bag. The samples were collected from mid-stream, mid-depth and sealed. The samples were then placed in a cooler on ice packs and immediately delivered to the University of New Hampshire Jackson Estuarine Laboratory (JEL).

Fecal Samples

Fecal samples were collected from the Great Bay Estuary watershed. The samples were collected from known sources using a sterile Whirlpak™ bag and immediately delivered to JEL. All of the fecal samples collected for this study were added to the Great Bay Source Species database at JEL.

Precipitation Data

Precipitation data was taken from the Durham weather station. The station is located in the Great Bay Estuary watershed and is maintained by the University of New Hampshire. The Durham Station precipitation data are sent to the DES Shellfish Program each month as daily precipitation totals and are entered into an Excel spreadsheet.

Laboratory and Analytical Methods

Detection of Fecal Coliforms and E. coli

Appropriate volumes of water samples were filtered to give at least 20 colonies on agar plates, where possible. The membrane filters were rolled onto mTEC agar in petri

dishes. Plates were inverted and incubated at 44.5±0.2 °C for 24 hours (USEPA, 1986). Fecal coliforms were enumerated by counting the yellow colonies after the incubation period, and *E. coli* was enumerated by counting the yellow colonies on the plate following incubation of the filter on urea substrate (Jones and Bryant, 2002).

For each sample/site, yellow colonies from the best dilution (10-30 readable colonies) were counted and recorded as fecal coliforms (Rippey et al., 1987). The yellow/yellow brown colonies remaining on the membrane filter after incubation on urea substrate were recorded as confirmed *E. coli* colonies.

Isolates Selected for Ribotyping

This study was restricted to ribotyping analysis for 300 isolates based on funding limitations. Ten isolates per sample were collected and ribotyped in accordance with the *Quality Assurance Project Plan* (Landry, In review). A subset of 30 samples from all of the project samples were selected for ribotyping analysis and selection was based on samples that exceeded state water quality standards.

Sample Processing

The procedures used for ribotyping *E. coli* isolates for this study have been used previously (Jones and Landry, 2003 and Jones, 2002) and are based to a large extent on those of Parveen et al. (1999). The procedures are also documented in Landry (In Review). *E. coli* isolates were stored in cryovials at -80°C and re-cultured onto trypticase soya agar (TSA). Some of the stored isolates could not be re-cultured. Cultures on TSA were incubated overnight at room temperature (~20°C). Some of the resulting culture was transferred to duplicate cryovials containing fresh glycerol/DMSO cryo-protectant media for long-term storage at -80°C.

A RiboPrinter was used to process *E. coli* cultures for ribotype determinations. After preparation of the samples, the automated process involved lysing cells and cutting the released DNA into fragments via the restriction enzyme EcoR1. These fragments were separated by size through gel electrophoresis and then transferred to a membrane, where they were hybridized with a DNA probe and mixed with a chemiluminescent agent. The DNA probe targeted 5S, 16S and 23S ribosomal RNA genes. A digitizing camera captured the light emission as image data, from which the system extracted a RiboPrint® pattern. This pattern could be compared to others in the RiboPrinter database for characterization and identification based on densiometry data, although our approach has conformed to other ribotyping studies in using banding patterns instead as the basis for comparing patterns.

Band Identification

The images were transferred from the RiboPrinter into GelComparII (Applied-Maths) analytical software. The bands in lanes containing the standard were labeled and

entered into the memory for optimization of gel pattern images. The densiometry data were processed for band identification. The ribopattern data for each separate water sample isolate were then selected for identification of source species.

Source Species Databases

There were two source species databases used to analyze ribopatterns from water samples. The first was the local Great Bay database that included 266 isolates from 19 source species, including septage and wastewater. The other was the New Hampshire database that included 808 isolates, including all of those from the Great Bay database, from 31 source species.

Table 2 Local Great Bay Estuary and New Hampshire source species databases.

Source species category	Source species	# of Isolates	
		Local Great Bay	New Hampshire
HUMAN	septage	10	16
	wastewater	53	115
	humans	59	82
PETS	cat	2	21
	dog	12	41
LIVESTOCK	alpaca	3	3
	Buffalo	6	6
	chicken	8	11
	cow	53	56
	goat	4	4
	horse	20	34
	oxen	5	5
	sheep	2	2
WILDLIFE	coyote	10	29
	deer	-	93
	mouse	-	2
	muskrat	-	12
	otter	-	14
	raccoon	4	84
	rabbit	-	27
	red fox	4	27
	skunk	-	5
AVIAN SPECIES	cormorant	-	12
	duck	-	15
	geese	3	42
	gull	-	28
	pigeon	5	5
	robin	-	4
	sparrow	-	3
	starling	-	3
	Wild turkey	3	7
	Total	266	808

Data Analysis

All data were analyzed with GelComparII software on a Dell computer, where the source species databases were also stored. Hard copies of ribotype patterns and similarity coefficients for the unknown and its most closely related source species were printed for interpretation. Interpretation and accompanying graphical representations of the data were done using MS Excel on Macintosh computers.

Optimization was set at 1.56% and band position tolerance was set at 1.00%. Both of these parameters were used to adjust the ability to differentiate between bands for the degree of accuracy desired, and also to compensate for possible misalignment of homologous bands caused by technical problems.

Similarity indices were determined using Dice's coincidence index (Dice, 1945) and the distance among clusters calculated using cluster analysis. The source species profile with the best similarity coefficient at the prescribed set of optimization and tolerance settings was accepted as an indication of the possible source species for the water sample isolate. For this study, the predetermined threshold similarity index that was considered to be a minimum value for identifying source species was 90% for comparisons to the source species databases. The identification of the source species was considered successful if the value calculated for a water isolate was equal to or greater than the threshold value; if the calculated value was below the threshold similarity index, the water sample isolate was considered to be of unknown origin. Thus, the results of the identifications reported are less than completely accurate (0% tolerance and 100% similarity).

Results and Discussion

Precipitation

Precipitation data were tabulated from the Durham weather station for each of the sampling dates and the previous 48-hour time periods (Table 3). Four of the sampling days were designated as dry weather and three as wet weather.

Table 3 Precipitation as recorded by the Durham, NH weather station.

			Rainfall amount (in.)[1]			
Date	**Weather Condition**	**48 h Total**	**Sample day**	**Previous day**	**2 Days prior**	**Sample Day Comments**
3/22/04	dry	0.44	0	0.23	0.21	Current weather partly cloudy, windy.
4/14/04	wet	1.67	0.12	1.55	0.00	Current weather rain, overcast.
4/20/04	dry	0.02	0	0	0.02	Current weather sunny.
5/4/04	wet	1.04	0.63	0.41	0	Current weather windy, cloudy. Start of last rain 5/3/04 at 12:30.
5/18/04	wet	0.56[2]	0.44	0	0.56	Current weather overcast. Light rain shower overnight, ~5am.
5/19/04	dry	0.44	0	0.44	0	Current weather overcast and breezy.
5/20/04	dry	0.44	0	0	0.44	Current weather sunny, warm.

[1] Precipitation data are recorded as 5 pm previous day through 5 pm date listed

[2]Precipitation from 5/18/04 was not added into "48 h total" because it fell after the sample collection.

Bacteria Concentration Data

E. coli and fecal coliform concentrations for each sample were measured as part of this study. The following tables show the *E. coli* results for the sites based on the condition of concern. Table 4 shows the results for the seven sites that were monitored during dry weather, the condition of concern, and Table 5 shows the results for sampling during one wet weather date. Two sites were sampled during dry and wet weather, GBPS001 (Table 6) and GBPS063 (Table 7). The *E. coli* and fecal coliform results for all sites are presented in Appendices B and C, respectively.

Table 4 *E. coli* concentrations (cfu/100 mL) during dry weather conditions in Spring 2004. Bolded samples were selected for ribotyping

Site	3/22	4/20	5/19	5/20
BLMPS017	no flow	4	**202**	**35**
GBPS014	1	2	**132**	**26**
GBPS044	2	**23**	**58**	11
GBPS064	**100**	**36**	**2310**	**290**
GBPS082	**1510**	**100**	70	**530**
ULBPS028	**14**	**13**	162	**3800**
WINPS001	8	1	**100**	**1800**

Table 5 *E. coli* concentrations (cfu/100 mL) during wet weather conditions on May 18, 2004. Bolded samples were selected for ribotyping

Site	5/18/2004
BLMPS017	**40**
GBPS014	**24**
GBPS044	**50**
GBPS064	176
GBPS082	12
ULBPS028	**1270**
WINPS001	**20**

Table 6 Site GBPS001 *E. coli* concentrations (cfu/100 mL) during dry and wet weather conditions in Spring 2004. Bolded samples were selected for ribotyping

Date	Weather	*E. coli* (cfu/100ml)
3/22	dry	6
4/14	wet	**38**
4/20	dry	8
5/4	wet	**600**
5/18	wet	154
5/19	dry	**222**
5/20	dry	88

Table 7 Site GBPS063 *E. coli* concentrations (cfu/100 mL) during dry and wet weather conditions in Spring 2004. Bolded samples were selected for ribotyping

Date	Weather	*E. coli* (cfu/100ml)
3/22	dry	**18**
4/14	wet	**56**
5/4	wet	**760**
5/18	wet	62
5/19	dry	**800**
5/20	dry	172

Selection Process for Ribotyping

Using an upper limit of 30 samples for ribotyping, the sample selection process was divided into two phases during the sample collection period. The first subset (n=12) of samples selected for ribotyping was based on a data set from sampling dates 3/22, 4/14, 4/20 and 5/4. The selection was largely based on concentration thresholds of State water quality standards. The standard for shellfish waters is based on a fecal coliform maximum of 14 MPN/100 mL and the surface water standard is based on an *E. coli* maximum of 406 cfu/100 mL.

The second phase occurred after all the sample collection was completed. The remaining 18 samples were selected from the 5/18, 5/19 and 5/20 sample collection dates. The first six of the 18 were selected based on exceeding an *E. coli* threshold of 406 cfu/100 mL. The next eight samples were selected based on the sampling sites that had not been previously represented in the first data subset. The next two samples were selected to increase the ribotyping samples for site GBPS044 from one to three. The last two samples were selected from two sites that have been difficult to determine the pollution sources in the past (GBPS001) and particularly high bacteria (GBPS064).

Table 8 lists the 30 samples that were chosen for ribotyping analysis by sites. At least 3 samples per site were selected. Tables 4 through 7 also identify the samples that were selected for ribotyping analysis and they are shown in bold type.

Fecal Material Collection

Fecal material was collected on May 12, 2004 from various locations in the watershed. Fecal material from the following animals were collected and delivered to JEL: horse, chicken, ox, goose, dog and human (municipal sewer line). JEL processed the material and isolated bacteria (*E. coli*). The isolates were ribotyped and these results were added to the ribopattern databases and included in the analysis of the unknown isolates collected in the surface water.

Table 8 Samples selected for ribotyping analysis listed by site.

Site	Sampling date	Weather condition
BLMPS017	5/18/2004	wet
BLMPS017	5/19/2004	dry
BLMPS017	5/20/2004	dry
GBPS001	4/14/2004	wet
GBPS001	5/4/2004	wet
GBPS001	5/19/2004	dry
GBPS014	5/18/2004	wet
GBPS014	5/19/2004	dry
GBPS014	5/20/2004	dry
GBPS044	4/20/2004	dry
GBPS044	5/18/2004	wet
GBPS044	5/19/2004	dry
GBPS063	3/22/2004	dry
GBPS063	4/14/2004	wet
GBPS063	5/4/2004	wet
GBPS063	5/19/2004	dry
GBPS064	3/22/2004	dry
GBPS064	4/20/2004	dry
GBPS064	5/19/2004	dry
GBPS064	5/20/2004	dry
GBPS082	3/22/2004	dry
GBPS082	4/20/2004	dry
GBPS082	5/20/2004	dry
ULBPS028	3/22/2004	dry
ULBPS028	4/20/2004	dry
ULBPS028	5/18/2004	wet
ULBPS028	5/20/2004	dry
WINPS001	5/18/2004	wet
WINPS001	5/19/2004	dry
WINPS001	5/20/2004	dry

Ribotyping Success

There were nearly 600 isolates for the total of 30 samples chosen for ribotyping. After biochemical testing of isolates for confirmation as *E. coli* , 279 confirmed *E. coli* isolates were chosen for ribotyping (Table 9) to represent the selected samples in Table 8. Further testing in the RiboPrinter revealed 20 more isolates that did not appear to be *E. coli*, leaving a total of 259 (93%) isolates for source species identification analysis. The sample ribotypes were analyzed first with the local Great Bay source species database (Table 2) and source species for 111 of the 259 isolates (43%) were identified. The sample ribotypes were then analyzed using the New Hampshire source species database, that included all ribotypes from the Great Bay database, and source species for 156 (60%) isolates were identified. All results discussed below are from analysis using the full New Hampshire database.

In some instances there were less than 10 isolates per sample to ribotype. There were several reasons for this situation. Some of the isolates that appeared to be *E. coli* based on mTEC colony reactions with urea did not pass all biochemical reactions that typify *E. coli*. Other species that passed biochemical tests gave ribopatterns that did not match well with *E. coli* patterns in the RiboPrinter database, and were more like other bacterial species. In a few cases too few colonies were available from water samples either because of low concentrations or use of dilutions that gave less than 10 colonies.

Table 9 Ribotyping success and *E. coli* concentrations at study sites.

Site	Geometric mean *E. coli* cfu/100 ml	Number of samples	Total isolates	Usable ribotypes	Identified ribotypes GB data	Identified ribotypes GB & NH data
BLMPS017	33	3	30	29	20	22
GBPS001	61	3	30	26	11	18
GBPS014	11	3	31	31	6	13
GBPS044	17	4	25	24	13	16
GBPS063	137	4	38	35	15	20
GBPS064	212	4	40	35	16	19
GBPS082	146	3	30	28	11	15
ULBPS028	170	4	34	33	12	21
WINPS001	31	3	21	18	7	12
Overall mean	61					
	Total	**31**	**279**	**259**	**111**	**156**

Source Species Identification

The ribotyping results for each site were summarized for all sample dates (Table 10). There were 18 different source species identified at all of the sites, including humans and wastewater as separate sources. The number of different species identified at each site ranged from 4 for GBPS-044, 064 AND 082, to 9 for BLMPS017. However, some of the identified source species were only identified once (deer, goat, horse, otter, rabbit, sparrow, wild turkey) and are considered insignificant sources. The most commonly identified source species were oxen (29 isolates), dog (26), wastewater (21), cow (19), goose (18), chicken (12), coyote and raccoon (7), fox (5) and cat (3). Clearly there are several source species that were much more commonly identified than others and probably represent sources of greater concern.

Table 10 Source species identified for *E. coli* isolates using a database of New Hampshire ribotypes.

Site name	# of samples	Isolates identified	cat	chicken	cow	coyote	deer	dog	fox	goat	goose	horse	human	otter	oxen	rabbit	raccoon	sparrow	turkey	ww[1]
BLMPS017	3	22	0	3	1	0	0	***6***	1	0	1	0	1	0	***5***	0	0	1	0	3
GBPS001	3	18	0	***4***[2]	1	2	0	0	0	0	2	0	0	1	3	1	0	0	0	***4***
GBPS014	3	13	2	2	0	0	1	***3***	2	0	0	0	0	0	0	0	3	0	0	0
GBPS044	3	16	0	0	0	0	0	***5***	0	0	4	0	1	0	***6***	0	0	0	0	0
GBPS063	4	20	0	2	***4***	3	0	0	0	0	1	1	0	0	***7***	0	0	0	0	2
GBPS064	4	19	0	0	***11***	0	0	3	0	0	0	0	0	0	3	0	0	0	0	2
GBPS082	3	15	0	0	0	0	0	0	2	0	***9***	0	0	0	0	0	2	0	0	2
ULBPS028	4	21	1	1	1	2	0	***7***	0	0	0	0	0	0	3	0	1	0	0	5
WINPS001	3	12	0	0	1	0	0	2	0	1	1	0	0	0	2	0	1	0	1	***3***
	30	156	3	12	19	7	1	26	5	1	18	1	2	1	29	1	7	1	1	21

[1] ww=wastewater

[2]Numbers in italics indicate the most numerous source species per site.

The most common source species at each site is an important observation that can help to direct pollution source elimination responses. In previous MST studies conducted in New Hampshire's Seacoast, results have shown some clearly dominant sources for some sites whereas other sites have had a variety of source species with no clear source to address. Some of the sites sampled in this study had clearly dominant source species (Table 11). For example, there were 11 cow isolates out of 19 total isolates at GBPS064, and 9 goose out of 15 total isolates at GBPS082. Oxen at GBPS063 and dogs at ULBPS028 were also more numerous than other source species at those sites. The most common source species at each of the other sites are also listed in Table 11. Overall, dogs (3 sites), wastewater (2 sites) and oxen (2 sites) seemed to be the most commonly dominant source species for sites. Others included chickens, cows and geese.

Table 11 Most common source species and types at each site.

Site Name	Isolates identified	Most common source species	Source species types				
			Human	Pets	Wild animals	Livestock	Birds
BLMPS017	22	dogs	4	6	1	9	2
GBPS001	18	chickens, wastewater	4	0	4	8	2
GBPS014	13	dogs	0	5	6	2	0
GBPS044	16	oxen	1	5	0	6	4
GBPS063	20	oxen	2	0	3	14	1
GBPS064	19	cows	2	3	0	14	0
GBPS082	15	geese	2	0	4	0	9
ULBPS028	21	dogs	5	8	3	5	0
WINPS001	12	wastewater	3	2	1	4	2
TOTAL	**156**		**23**	**29**	**22**	**62**	**20**

Analysis of results using types of source species helps to categorize sources according to different management approaches for elimination. Livestock, including oxen, cows, horses, goats and chickens, were the most numerous source species type and the most dominant type at six of the nine study sites (Table 11). Pets, including dogs and cats, were the next most commonly identified source type and pets (dogs) were the most dominant source species at ULBPS028. Even though dogs were the most common source species at GBPS014, wild animals, specifically raccoon, fox and deer were the most commonly identified source type. 'Humans', including wastewater and humans, were identified at all sites except GBPS014, but were not the dominant type at any of the sites. Birds were the most common source species type at GBPS082 because of the prevalence of goose isolates.

There were few obvious temporal patterns for the incidence of source species at sites. The main temporal observation was that source species at several sites were identified on only one day. For example, chickens were only identified at BLMPS017 on May 20, 2004. Most of the identified dog and oxen isolates were sampled at GBPS014 on May 18, 2004. At GBPS063, most of the identified oxen isolates were sampled on May 4, 2004. All of the identified oxen isolates at GBPS064 were sampled on May 20,

2004 while most of the cow isolates were sampled on March 22, 2004. Most of the geese isolates were sampled on March 22, 2004 at GBPS082, and most of the dog isolates identified at ULBPS028 were sampled on May 18, 2004.

Samples were collected under both dry and wet weather conditions (Table 3). Both weather conditions are represented in the samples selected for ribotyping (Table 8) except sites GBPS064 and 082 that had no wet weather samples ribotyped. *E. coli* concentrations were not consistently higher under either set of conditions. However, some source species, especially dogs, were identified more extensively under wet weather conditions at some sites. For example, most or all identified dog isolates were sampled on May 18, 2004 at GBPS014, GBPS044 and ULBPS028. Most of the identified oxen isolates were sampled on wet dates at GBPS-044 and 063. Other sites did not show any obvious trends relative to weather conditions.

Discussion

In this study, there were new source species ribopatterns added to the databases from fresh fecal samples, including oxen isolates that had not been included prior to this study. These and other ribopatterns from local source species helped to identify source species for many of the water sample isolates of unknown origin. This helps to support the approach of using local species to help identify significant sources at sites. However, the much larger and more diverse New Hampshire database added source species identifications for 45 more isolates, which gave 60% identification overall. Thus, a combination of approaches is a useful strategy for optimizing source identification. This is especially true for studies like this one where a variety of sites with diverse sources and spread around a relatively large area were investigated.

There were six source species that constituted 125, or >80% of the total 156 identified isolates. Oxen, dogs, wastewater, cows, geese and chickens were clearly the dominant overall source species. However, they were not identified evenly at all sites. Instead, each of these six sources was dominant at one or more sites, and each was absent at other sites. The dominance of one or more source species at sites is a useful result that helps to direct resource allocation for pollution source elimination.

There were no obvious temporal trends for source species at sites that suggested seasonal occurrences or practices such as springtime spreading of manure. However, the temporal intensity of sampling was probably inadequate to address such questions, and this was not the main purpose of the study.

One of the sampling criteria was to sample under both wet and dry weather conditions to determine if source species differed based on weather and runoff conditions. Even though *E. coli* concentrations in samples collected under wet weather conditions were not always greater than samples collected under dry conditions, previous studies have shown runoff at most of these sites to be significant and a large contribution to the increased flow observed during wet weather at these sites (C. Nash, personal

communication). Thus, the loading of bacteria from these sites during wet weather is probably much greater under runoff conditions, and sources identified under these conditions may be more significant. The observation of dog (and oxen) isolates being identified at some sites predominantly under wet weather/runoff conditions is consistent with the suspected mode of transport of dog feces to surface waters.

The results from this MST study are the first for most of the investigated sites. A previous study in the Bellamy River showed livestock to be a dominant type of source in the area (Jones, 2002). The results were not expected at that time and the study recommended follow-up studies to confirm results. The results of the present study show livestock species, including oxen and cows, to be the most prevalent type of source at BLMPS017. These results confirm a probable site and source species for some of the livestock sources identified in the previous study.

The source species identification results also show a much greater prevalence of wastewater isolates compared to human isolates. This could be attributed to a greater number of database isolates for wastewater compared to humans, but the numbers were similar for these sources in both databases. A more likely reason for this difference in occurrence in surface waters is that *E. coli* isolates found in wastewater are those that have survived environmental conditions outside of the human intestine. These isolates may be more capable of survival in surface waters compared to isolates collected directly from human feces, where the predominant isolates would be those most capable of growth in the human gastrointestinal environment. McLellan (2004) reported results that suggested a limited number of persistent *E. coli* occurred in contaminated storm water from Milwaukee, WI. Another reason may be related to observations that isolates from individual humans tend to have highly similar patterns, so that despite a large number of isolates from humans in the database, the patterns do not have enough diversity to represent the large human population surrounding Great Bay Estuary. The results support the use of wastewater as an important source to be included in ribotyping databases.

Management Recommendations

Management recommendations focus on the identification, reduction and/or elimination of sources that are dominant and/or more controllable. As stated earlier in this report, the most common source species at each site is an important observation that can be useful to help direct pollution source elimination responses. The dominant source species for the study area were livestock, humans, wildlife, and pets. Additionally, some sources, such as humans, are assumed to be potentially more controllable than non-human sources (Schueler and Holland, 2000). Therefore, human sources will be addressed at sites where it may be possible to implement controls after an investigation of the situation is conducted. Other information such as previous water quality studies, local land use, and field observations at the time of sampling were also considered as the recommendations were developed. Recommendations are provided below by source species and site. Local agencies and officials that could potentially provide assistance to DES/NHCP are also listed.

Humans

The priority areas for investigating human sources include sites where wastewater and/or human source species results were dominant or prevalent. These sites include BLMPS017, GBPS001, ULBPS028, and WINPS001. Further investigations should focus on septic systems and/or wastewater treatment infrastructure as described below on a site-by-site basis with the participating investigating agency noted.

1. GBPS001 and ULBPS028: Septic systems are the primary means of wastewater treatment in these subwatersheds. Potentially failing systems may contribute to bacterial contamination at these sites. Work with DES and municipal health officials to further investigate potential septic system failures using on-site investigations. Address failures as needed through septic system rehabilitation, owner education, and enforcement (if necessary). Previous investigations by DES revealed a failing septic system in the GBPS001 subwatershed which was replaced prior to this study.

2. BLMP017: Most homes in this subwatershed are connected to the municipal wastewater treatment system. Wastewater treatment infrastructure is located throughout the subwatershed. It is possible that leaking infrastructure may result in bacterial contamination to the surface water. Work with DES and City of Dover to investigate potential exfiltration from wastewater treatment infrastructure.

3. WINPS001: Determine the type of wastewater treatment service in the vicinity of this site and proceed accordingly to investigate potential sources.

Livestock

Investigate the possibility of ox, cow, and chicken as the source species potentially related to livestock barns and manure spreading operations in the subwatersheds of the following sites BLMPS017, GBPS001, GBPS044, GBPS063, GBPS064, and ULBPS028.

1. Work with the Natural Resource Conservation Service to identify livestock barns and farm fields that use manure as fertilizer. Provide guidance to operations managers for best management practices.

2. Distribute educational materials about the proper handling of livestock waste and animal fertilizers as appropriate.

Wildlife

Generally, wildlife source species were not dominant at many sites in the study area; however, at GBPS082, isolates from geese were more numerous than those from other source species. While some wildlife source species may be difficult to control or manage, it is possible, depending on the nature and extent of the problem, that management actions for geese could be readily implemented.

1. Conduct surveys of the area to determine if domestic geese or wild geese are present in significant numbers. Investigate the possibility that residents or visitors to the area are feeding and thereby attracting wild geese. If such is the case, work with the local health officer to provide residents with educational information about ways to reduce nuisance species.

Pets

Dogs were reported to be a dominant or prevalent source species at several sites including BLMPS017, GBPS014, GBPS044, and ULBPS028. To address this issue, DES could work with NHCP and local health officers to conduct public outreach to promote proper disposal of pet waste.

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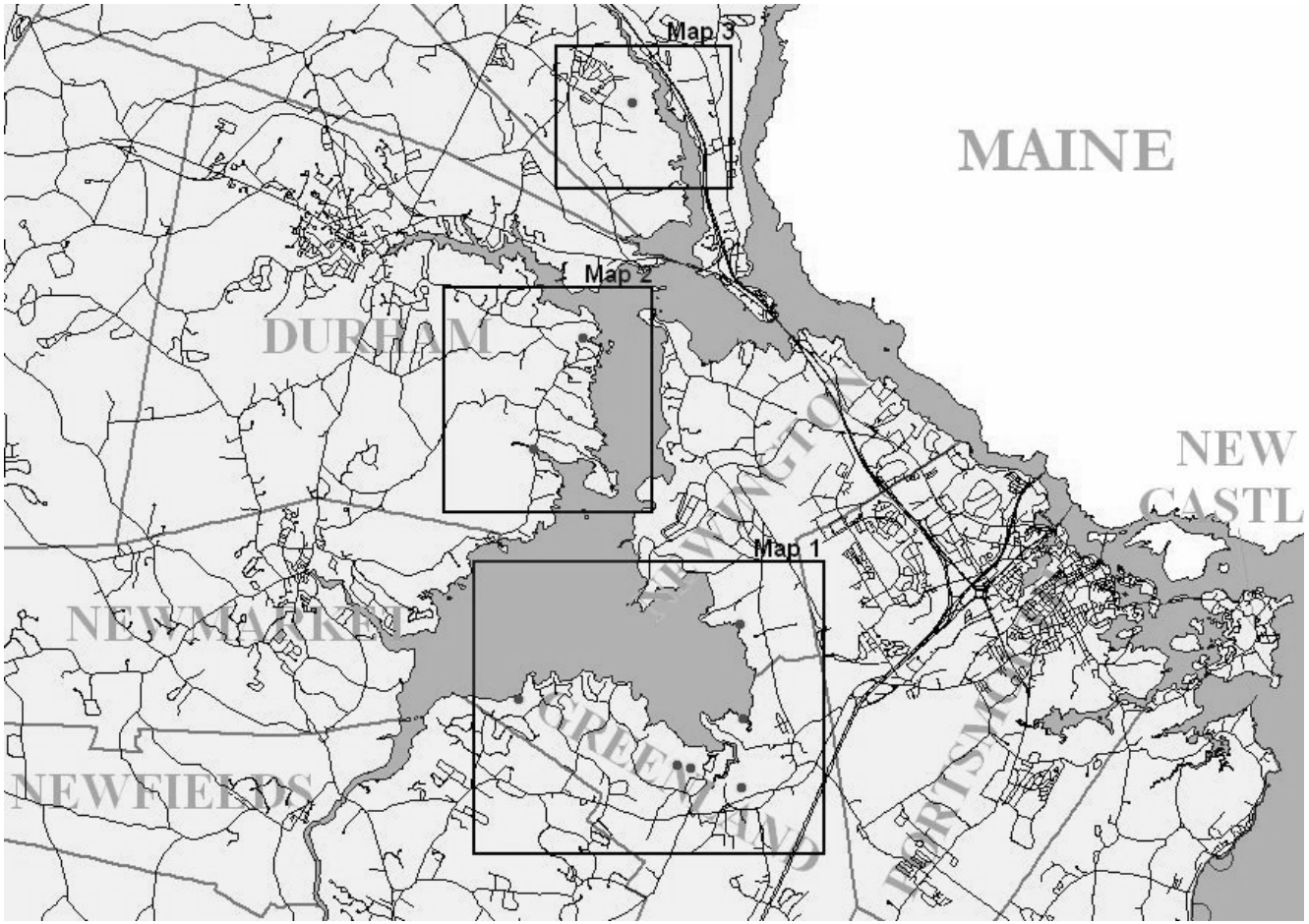
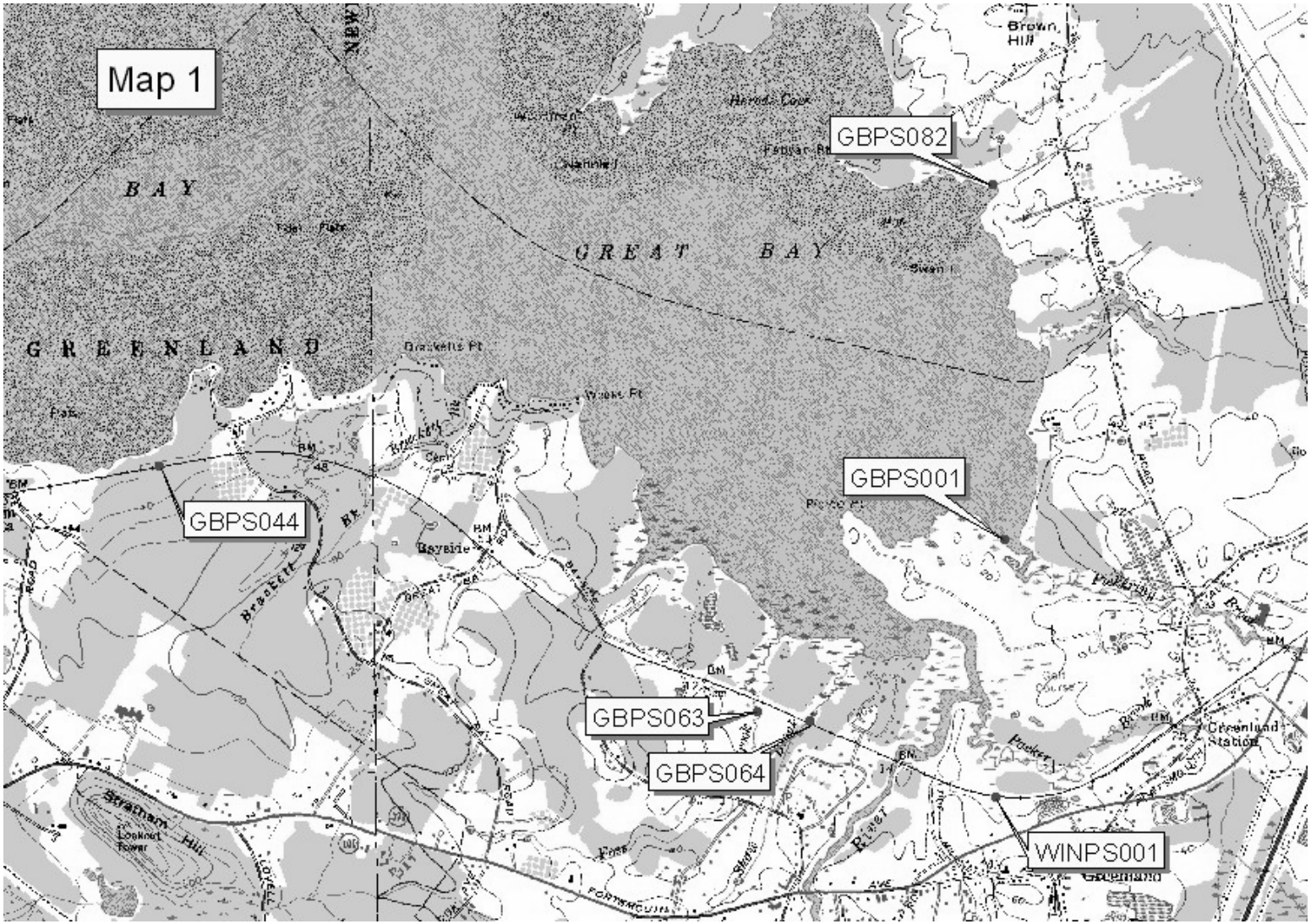
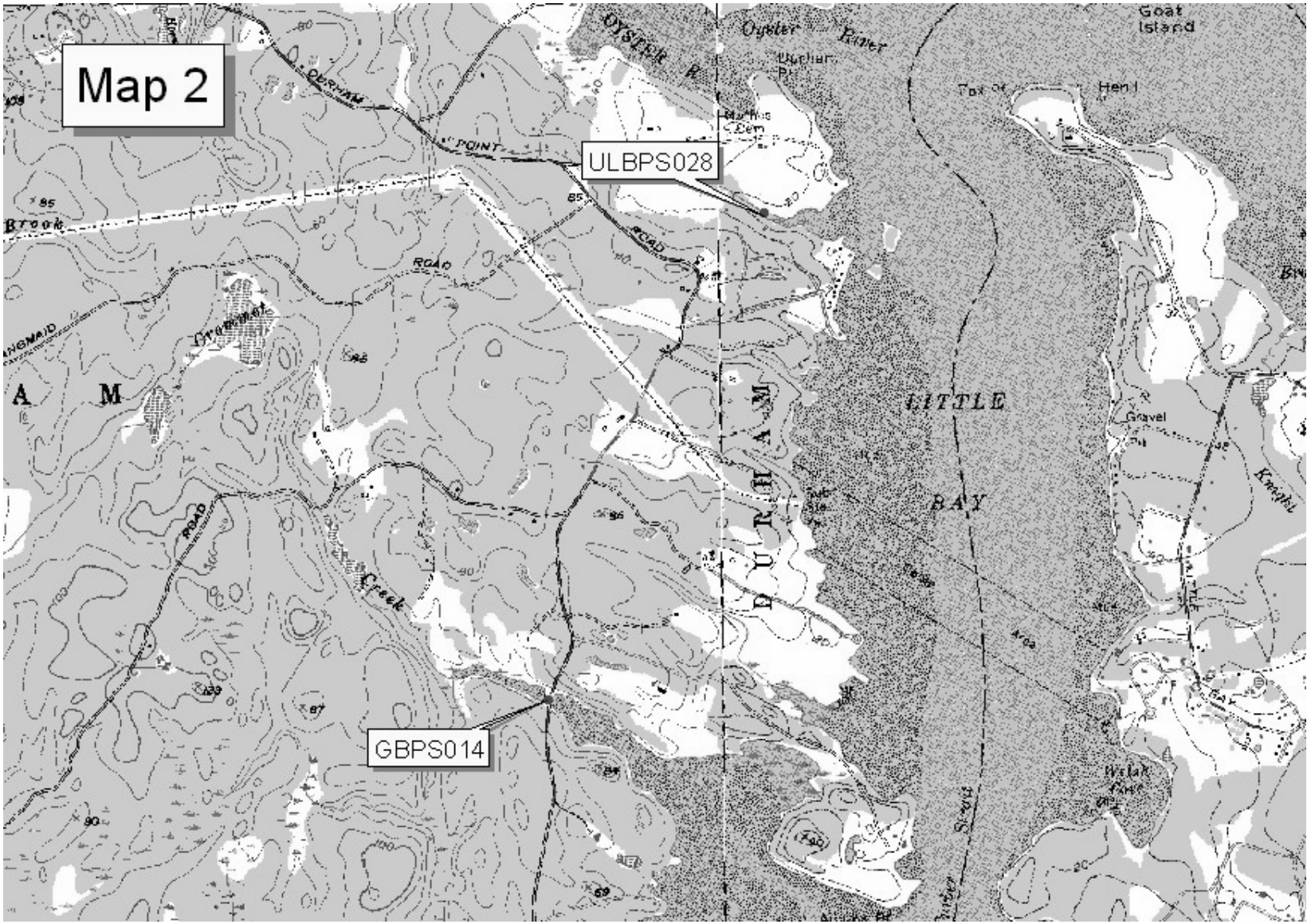
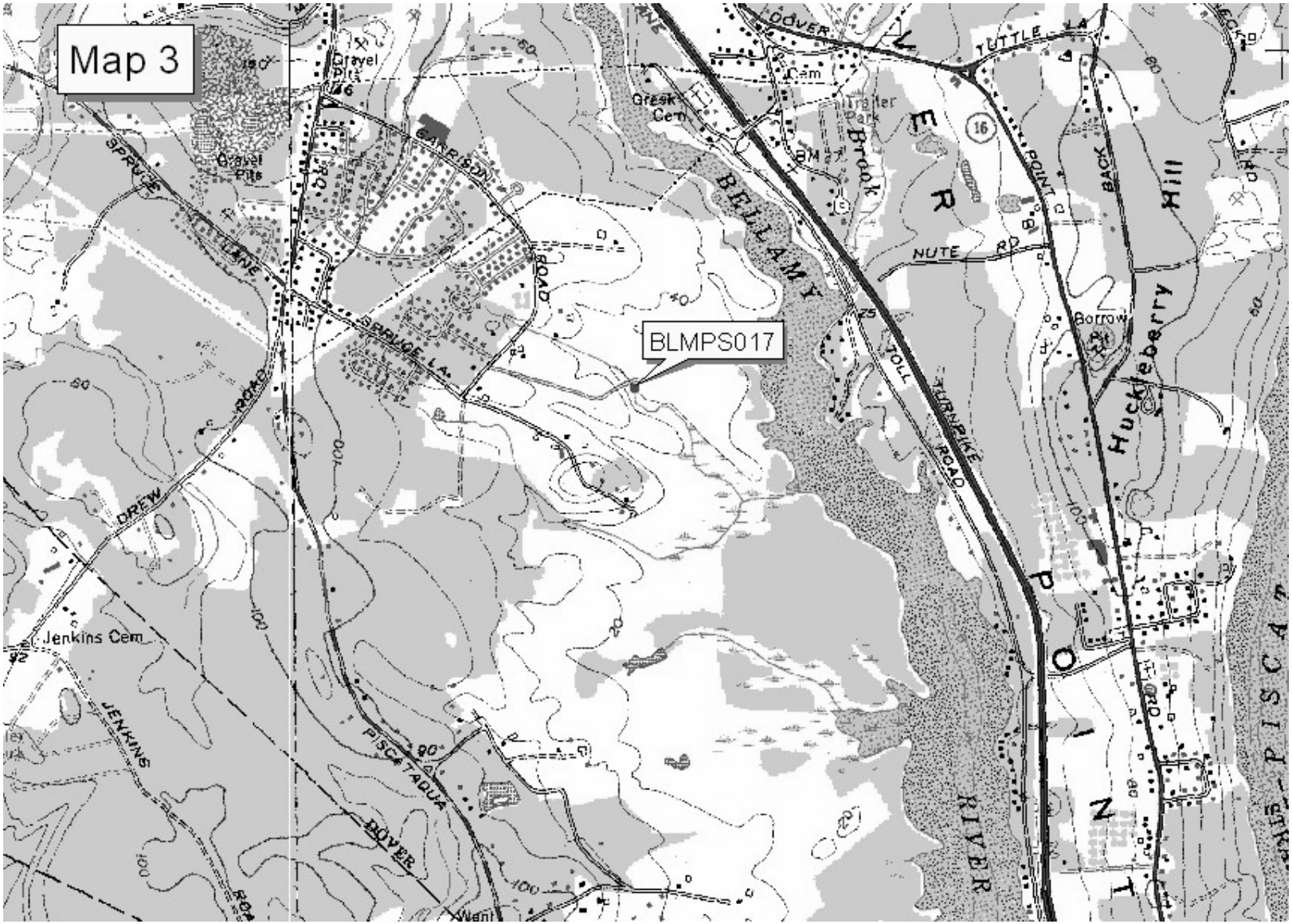
Appendix A

Site location maps for Great Bay Estuary sampling sites (Source: Matthew Wood, DES Shellfish Program).


Map 3
Map 2
Map 1
MAINE
DURHAM
NEW
CASTL
NEWMARKET
NEWFIELDS


Map 1
GBPS082
GBPS044
GBPS001
GBPS063
GBPS064
WINPS001
BAY
GREAT BAY
GREENLAND
Brown Hill
Bayside
Greenland Station
Strathern Lookout Tower Hill


Map 2
ULBPS028
GBPS014
OYSTER R.
Oyster River
Goat Island
Hen I
LITTLE
BAY
DURHAM
Gravel
Creek
ROAD
POINT


Map 3
BLMPS017
BELLAMY
RIVER
POINT
Huckleberry Hill
Brook
Gresk Cem
Jenkins Cem
Gravel Pits
Borrow
TUTTLE LA
POINT RD
BACK RD
NUTE RD
TOLL TURNPIKE ROAD
GARRISON ROAD
SPRUCE LANE
SPRUCE LA
DREW ROAD
JENKINS ROAD
PISCATAQUA
DOVER
PISCATAQUA

Appendix B

***E. coli* concentrations (cfu/100mL) and geometric means for Great Bay Estuary sites during Spring 2004.**

Date	Weather	BLMPS017	GBPS001	GBPS014	GBPS044	GBPS063	GBPS064	GBPS082	ULBPS028	WINPS001
3/22/2004	dry	no flow	6	1	2	18	100	1510	14	8
4/14/2004	wet		38			56				
4/20/2004	dry	4	8	2	23		36	100	13	1
5/4/2004	wet		600			760				
5/18/2004	wet	40	154	24	50	62	176	12	1270	20
5/19/2004	dry	202	222	132	58	800	2310	70	162	100
5/20/2004	dry	35	88	26	11	172	290	530	3800	1800
Geometric mean	All weather	33	61	11	17	137	212	146	170	31
Geometric mean	Dry	30	31	9	13	135	222	274	103	35
	Wet	40	152	24	50	138	176	12	1270	20

Appendix C

Fecal coliform concentrations (cfu/100mL) and geometric means for Great Bay Estuary sites during Spring 2004.

Date	Weather	BLMPS017	GBPS001	GBPS014	GBPS044	GBPS063	GBPS064	GBPS082	ULBPS028	WINPS001
3/22/2004	dry	no flow	13	1	2	20	110	1560	16	8
4/14/2004	wet		42			58				
4/20/2004	dry	4	11	3	26		41	104	14	2
5/4/2004	wet		656			760				
5/18/2004	wet	56	160	64	80	68	188	112	1280	20
5/19/2004	dry	236	224	136	80	820	2400	70	162	104
5/20/2004	dry	41	92	28	11	184	328	530	4000	1800
Geometric mean	All weather	38	75	15	21	144	232	232	179	36
Geometric mean	Dry	34	41	10	15	145	244	279	110	42
	Wet	56	164	64	80	144	188	112	1280	20